Micro-volume spectroscopy: thinking outside the cuvette

NanoDrop Technologies has developed a patented sample-retention system that allows scientists to perform absorbance and fluorescence analysis with only 1 μl of sample. The NanoDrop® ND-1000 spectrophotometer and the NanoDrop® ND-3300 fluorospectrometer are the first microvolume instruments to use the inherent surface tension properties of the sample to create its own containment, eliminating the need for cuvettes, capillaries and microcells.

Microvolume spectroscopy

As methods evolve toward the use of ever smaller amounts of material for analysis, strategies for absorbance and fluorescence measurement with minimal sample consumption have become paramount in importance. Conventional methods are impractical given the limited sample volumes produced by techniques such as laser-capture microdissection. Using fiberoptic technology and the inherent surface-tension properties of liquid samples, NanoDrop Technologies’ microvolume instrumentation can accurately quantitate a wide range of biomolecules in volumes as small as 1 μl. Here we describe the new microvolume spectrophotometric and fluorometric systems, and how measurements are made using the NanoDrop ND-1000 spectrophotometer and the NanoDrop ND-3300 fluorospectrometer.

Sample-retention technology

The NanoDrop sample-retention system allows absorbance and fluorescent measurements to be performed without traditional containment devices such as cuvettes or capillaries. The system uses inherent surface tension to hold a 1-μl sample in place between two optical surfaces during the measurement cycle.

Microvolume absorbance measurements

To make a measurement, 1 μl of sample is pipetted directly onto the lower optical (measurement) surface (Fig. 1a). An upper optical surface automatically engages the sample, using surface tension to form a liquid column of mechanically controlled path length (Fig. 1b). Once the measurement is complete, the user simply cleans both optical surfaces with a standard laboratory wipe to prepare for the next sample.

The ultraviolet-to-visible wavelength range of 220 nm to 750 nm provides a versatile platform for absorbance analysis of various...
chromophores, including nucleic acids and proteins. The variety of modules within the ND-1000 software combines diversity of possible measurements with ease of use. The Microarray module is a good example of how the software is designed to quickly provide investigators with critical information. After the ten-second measurement cycle is complete, the module displays a full absorbance spectrum as well as the calculated concentrations of both the nucleic acid and the fluorescent label. Investigators can readily determine incorporation rates with only 1 μl of final probe, preserving most of the sample for array hybridization (Fig. 2).

By design, the NanoDrop ND-1000 spectrophotometer eliminates fixed containment devices such as cuvettes and capillaries, allowing automatic path length change from 1 mm to 0.2 mm during the same measurement cycle of a given sample. This allows for an unprecedented range of sample concentration to be assessed through absorbance spectroscopy (2 ng/μl to 3,700 ng/μl for dsDNA), essentially eliminating the need to perform dilutions.

**Microvolume fluorescence measurements**
Using the same sample retention technology, the NanoDrop ND-3300 fluorospectrometer performs full-spectrum fluorescent analysis of 1-μl samples without the use of cuvettes or capillaries. The lever arm of the ND-3300 fluorospectrometer is opened and 1–2 μl of sample is pipetted onto the optical surface. The lever arm then engages the upper surface of the sample to capture and hold the sample in place during the measurement cycle. Once complete, the user wipes the sample away using an ordinary laboratory wipe. Fluorophore excitation occurs from one of three solid-state light emitting diodes (LEDs): ultraviolet (UV), blue or white. The broad excitation range allows for the measurement of a wide range of common fluorophores without filter changes (Fig. 3a). The uniquely clean optics of the retention system and proprietary signal processing permit the unconventional use of the broad-spectrum, white LED. The broad excitation of the white LED can be used to excite several fluorophores, allowing for a multiple-emission profile from a single sample (Fig. 3b).

**Ultralow sample mass detection limit**
The small sample volume requirement of the NanoDrop ND-3300 fluorospectrometer allows significantly scaled-down fluorescent reaction volumes (2–10 μl) and therefore uses only a fraction of the sample consumed by cuvette- or plate-based fluorescence measurements. Even though the ND-3300 does not measure ultralow concentrations, it does lower the fluorescent detection limit of sample mass by more than an order of magnitude. For example, using the PicoGreen® dye (Invitrogen, Molecular Probes), the ND-3300 can detect as little as 2 pg dsDNA, whereas a cuvette or microplate PicoGreen assay would require at least 25–50 pg dsDNA for detection (Table 1). For those working with limited sample quantities, the ND-3300 allows measurements that are simply not possible using cuvette- or plate-based fluorescence spectroscopy.

**Conclusion**
NanoDrop Technologies’ new retention system not only enables quality absorbance and fluorescence measurements with only a minute fraction of the sample material needed to perform similar analysis on more traditional systems, but also provides a practical and easy-to-use alternative for all spectroscopy measurements.

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**Table 1 | PicoGreen detection limits**

<table>
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<tr>
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<th>NanoDrop ND-3300</th>
<th>Microplate reader</th>
<th>Cuvette-based fluorometer</th>
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<tbody>
<tr>
<td>Detection limit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>by sample mass</td>
<td>2 pg (in 2 μl)</td>
<td>50 pg (in 200 μl)</td>
<td>25 pg (in 1 ml)</td>
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<tr>
<td>Detection limit</td>
<td>1 pg/μl</td>
<td>0.25 pg/μl</td>
<td>0.025 pg/μl</td>
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